Development and validation of a simplified method to generate human microglia from pluripotent stem cells.

Journal: Mol Neurodegener

Publication Year: 2018

Authors: Amanda McQuade, Morgan Coburn, Christina H Tu, Jonathan Hasselmann, Hayk Davtyan, Mathew Blurton-Jones

PubMed link: 30577865

Funding Grants: Optimizing the differentiation and expansion of microglial progenitors from human pluripotent stem cells for the study and treatment of neurological disease.

Public Summary:
BACKGROUND: Microglia, the principle immune cells of the brain, play important roles in neuronal development, homeostatic function and neurodegenerative disease. Recent genetic studies have further highlighted the importance of microglia in neurodegeneration with the identification of disease risk polymorphisms in many microglial genes. To better understand the role of these genes in microglial biology and disease, we, and others, have developed methods to differentiate microglia from human induced pluripotent stem cells (iPSCs). While the development of these methods has begun to enable important new studies of microglial biology, labs with little prior stem cell experience have sometimes found it challenging to adopt these complex protocols. Therefore, we have now developed a greatly simplified approach to generate large numbers of highly pure human microglia. RESULTS: iPSCs are first differentiated toward a mesodermal, hematopoietic lineage using commercially available media. Highly pure populations of non-adherent CD43(+) hematopoietic progenitors are then simply transferred to media that includes three key cytokines (M-CSF, IL-34, and TGFbeta-1) that promote differentiation of homeostatic microglia. This updated approach avoids the prior requirement for hypoxic incubation, complex media formulation, FACS sorting, or co-culture, thereby significantly simplifying human microglial generation. To confirm that the resulting cells are equivalent to previously developed iPSC-microglia, we performed RNA-sequencing, functional testing, and transplantation studies. Our findings reveal that microglia generated via this simplified method are virtually identical to iPS-microglia produced via our previously published approach. To also determine whether a small molecule activator of TGFbeta signaling (IDE1) can be used to replace recombinant TGFbeta1, further reducing costs, we examined growth kinetics and the transcriptome of cells differentiated with IDE1. These data demonstrate that a microglial cell can indeed be produced using this alternative approach, although transcriptional differences do occur that should be considered. CONCLUSION: We anticipate that this new and greatly simplified protocol will enable many interested labs, including those with little prior stem cell or flow cytometry experience, to generate and study human iPS-microglia. By combining this method with other advances such as CRISPR-gene editing and xenotransplantation, the field will continue to improve our understanding of microglial biology and their important roles in human development, homeostasis, and disease.

Scientific Abstract:
BACKGROUND: Microglia, the principle immune cells of the brain, play important roles in neuronal development, homeostatic function and neurodegenerative disease. Recent genetic studies have further highlighted the importance of microglia in neurodegeneration with the identification of disease risk polymorphisms in many microglial genes. To better understand the role of these genes in microglial biology and disease, we, and others, have developed methods to differentiate microglia from human induced pluripotent stem cells (iPSCs). While the development of these methods has begun to enable important new studies of microglial biology, labs with little prior stem cell experience have sometimes found it challenging to adopt these complex protocols. Therefore, we have now developed a greatly simplified approach to generate large numbers of highly pure human microglia. RESULTS: iPSCs are first differentiated toward a mesodermal, hematopoietic lineage using commercially available media. Highly pure populations of non-adherent CD43(+) hematopoietic progenitors are then simply transferred to media that includes three key cytokines (M-CSF, IL-34, and TGFbeta-1) that promote differentiation of homeostatic microglia. This updated approach avoids the prior requirement for hypoxic incubation, complex media formulation, FACS sorting, or co-culture, thereby significantly simplifying human microglial generation. To confirm that the resulting cells are equivalent to previously developed iPSC-microglia, we performed RNA-sequencing, functional testing, and transplantation studies. Our findings reveal that microglia generated via this simplified method are virtually identical to iPS-microglia produced via our previously published approach. To also determine whether a small molecule activator of TGFbeta signaling (IDE1) can

be used to replace recombinant TGFbeta1, further reducing costs, we examined growth kinetics and the transcriptome of cells differentiated with IDE1. These data demonstrate that a microglial cell can indeed be produced using this alternative approach, although transcriptional differences do occur that should be considered. CONCLUSION: We anticipate that this new and greatly simplified protocol will enable many interested labs, including those with little prior stem cell or flow cytometry experience, to generate and study human iPS-microglia. By combining this method with other advances such as CRISPR-gene editing and xenotransplantation, the field will continue to improve our understanding of microglial biology and their important roles in human development, homeostasis, and disease.
